CLAIMS

We claim:

1. A method of sequencing a plurality of target nucleic acids each comprising a first domain and a adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing a plurality of hybridization complexes each comprising a target sequence and a sequencing primer that hybridizes to the first domain of said target sequence, said hybridization complexes attached to a surface of a substrate;

b) extending each of said primers by the addition of a first nucleotide to the first detection position using a first enzyme to form an extended primer; and

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primers.

2. A method according to claim 1 wherein said hybridization complexes are attached to microspheres distributed on said surface.

3. A method according to claim 1 wherein said sequencing primers are attached to said surface.

4. A method according to claim 1 wherein each of said hybridization complexes comprises said target sequence, said sequencing primer and a capture probe covalently attached to said surface.

5. A method according to claim 1 wherein each of said hybridization complexes comprises said target sequence, said sequencing primer, an adapter probe and a capture probe covalently attached to said surface.

6. A method according to claim 1 further comprising:

d) extending said extended primer by the addition of a second nucleotide to the second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primers.

7. The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

10. A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing a hybridization complex comprising said target sequence and a capture probe covalently attached to a microsphere on a surface of a substrate; and

b) determining the identity of a plurality of bases at said target positions.

11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.

12. A method according to claim 10 wherein said sequencing primer is said capture probe.

13. A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to the first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to the second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. The method according to claim 13 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

17. A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said enzyme; and

f) determining the identification of said second protected nucleotide.

18. A kit for nucleic acid sequencing comprising:

a) a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres distributed on said sites;

wherein said microspheres comprise capture probes;

b) an extension enzyme; and

c) dNTPs.

19. A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP.

20. A kit according to claim 18 wherein said dNTPs are labeled.

21. A kit according to claim 20 wherein each dNTP comprises a different label.